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GROWTH AND YIELD OF PROGENY FROM PLANTS
WHICH WERE TREATED WITH FERTILIZERS

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Growth and Yield of Progeny from Plants which were Treated with Fertilizers" submitted by Lin-Ying Chiang, in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Barley grain samples from five field fertilizer experiments, which showed significant response to nitrogen and/or phosphorus fertilizer, were sorted into size categories. Nitrogen fertilizer significantly increased the proportion of large seed and decreased that of small seed at four locations. Phosphorus fertilizer showed similar trends at most of the locations.

Sorted samples were planted in the field and greenhouse to determine the effects of seed size and parental fertilization on early plant growth and final yields. Large seeds produced significantly more vigorous seedlings, more healthy heads per unit area, fewer smutted heads, and greater grain and straw yields than did small seeds. Parental fertilization did not show any definite effect on early plant growth or on final yields.

It was assumed in this study that variation in the quantity of energy sources stored in the seed could account for the differences observed in the above studies. Determinations of two major seed components, namely crude protein (Kjeldahl-N \times 6.25) and total available carbohydrates (free sugars and residual available carbohydrates), were carried out. It was noted that nitrogen fertilization significantly increased Kjeldahl-N and percent free sugars, but reduced percent residual available carbohydrates.

Significant S \times N and S \times P interactions were observed in some locations, indicating that the response to variation in parental fertilization was not the same for all seed sizes. The strong N \times P interaction found in some cases indicated the interdependent relation between nitrogen and phosphorus fertilization.

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INTRODUCTION

There is no doubt that suitable fertilization usually improves plant growth and increases final yields. It has been reported also that improvement of feeding and malting qualities and increase in seed size of the barley crop may be brought about by fertilization. Some workers have shown that large seeds produced more vigorous seedlings, more tillers, and greater yield than did small seeds. The above information indicates that fertilization may increase seed size which, in turn may influence plant growth and yield. No attempt has been made to study this possibility or to find out whether, for a given size, seed from fertilized crops is superior to that from unfertilized crops.

Thus the present study included the following investigations:

1. To find out whether or not fertilization influences the seed size.
2. To determine whether or not this "parental fertilization"* influences the growth and yield of second generation plants.
3. To verify the previous observations concerning the influence of seed size on early growth and yield.
4. To determine whether there is any difference in chemical composition of seeds from various sources, namely large and small seeds from parents which received different fertilizers.

* Throughout this report, the term "parental fertilization" refers to fertilization of the crops from which the seeds evaluated in the study, were obtained.

REVIEW OF LITERATURE

The yield and quality of field crops depend upon uncontrollable and controllable factors. The former includes climatic factors (particularly sunlight, temperature and rainfall); the latter, such factors as genetic constitution which can be controlled through hybridization and seed selection, and soil condition which can be modified by adoption of better cropping systems along with proper fertilization.

Effects of fertilization and seed selection on quantity (yield and yield components), and quality (1000 kernel weight, growth potential and chemical composition of seed) of cereal crops, and chemical composition of seed in relation to biochemical aspects of subsequent growth will be reviewed.

Incidentally, non-genetic occurrence of loose smut will be included as well.

I. Effects of Fertilization

The effects of fertilization on yield and quality of cereal crops have been intensively studied by many workers. The fact that a response of a crop to a fertilizer depends on many factors, such as the level of other nutrients, environmental conditions, etc., so that no simple exact quantitative relation between supply and crop response can exist, has to be realized.

Alberta soils vary considerably in their nutrient status. Plants grown on soils in different parts of the province respond to fertilization with different nutrients because of the variation in available nutrient supply. The greatest responses in terms of plant growth and yield are obtained with nitrogen, phosphorus, and sulfur (University of

Alberta et al., 1959, p. 9 - 16). For the present study, the review will be confined mainly to nitrogen and phosphorus fertilizers.

A. On yield and yield components

Within a variety, yield of cereal crops is a function of three components: they are number of heads per unit area, number of kernels per head, and the average weight of kernel. The yield components vary according to variety (Frey, 1959a; 1959b; 1959c), location (Frey, 1959c; McFadden, 1964), date of seeding (Anderson and Hennig, 1964; Frey, 1959; Meredith, Olson and Rowland, 1942; Middleton, 1964), level of soil nitrogen (Anderson and Hennig, 1964; Frey, 1959b), and seeding rate (Demirlicakmak, Kaufmann and Johnson, 1964; Guitard, Newman and Hoyt, 1961; Meredith et al., 1942; Middleton, Hetebert and Murphy, 1964).

It has been shown that suitable fertilization with nitrogen and/or phosphorus increased the various yield components of cereal crops, which in turn increased the grain yield (Dubetz and Wells, 1965; Frey, 1952; Greaves and Pittman, 1946; Hall, 1905; Hobbs, 1953; Kopeczky, 1965; Larter, 1958; Martin and Mikkelsen, 1960; McBeath, 1959; McNeal and Davis, 1954; Rankin, 1946; Reisenauer and Dicken, 1961; Rohde, 1963; Sexsmith and Russell, 1963; Singh, 1964; Stanberry and Lowery, 1965).

Depending upon the stage of growth when nitrogen was applied, yield components were affected to different extents. For example, Garner (1949) reported that earlier nitrogen applications increased the number of spikes per plant while late applications increased the size of the kernel, but Wahhab and Hussain (1957) and McBeath (1959, p.41) found that nitrogen fertilization at seeding time increased the number of tillers per plant, number of mature heads per plant, number of grains per head, the weight of 1000 kernels and yield of grain per acre.

Black (1957) stated that grain size usually was not much reduced by deficiencies of nitrogen or phosphorus. Frey (1959b) and many others (Hall, 1905; Rankin, 1946; Wahhab and Hussain, 1957) showed that 1000 kernel weight was increased due to the suitable application of nitrogen fertilizer at the proper time. As quoted by McBeath (1959, p.4) it was reported by Colic et al. (1950) and Garner (1948) that the increase in the size and average weight of grain was produced by late nitrogen application at about the flowering stage, while Wahhab and Hussain (1957) and McBeath (1959, p.41) found that nitrogen fertilization at seeding time increased the 1000 kernel weight of cereal crops. However, an adverse effect was obtained when nitrogen was applied in excess (Reisenauer and Dickson, 1961). Atkins, Stanford and Dumenil (1955) working with barley on phosphorus-deficient soils in northwest Iowa, reported that phosphorus fertilizer increased the 1000 kernel weight. Singh (1964) showed that the 1000 kernel weight of wheat was increased by the application of nitrogen and/or phosphorus. Bauer and Vasey (1964) and Black (1957) reported that potassium fertilization increased the percentage of plump seed of barley, which resulted in the increase of 1000 kernel weight, whereas Widdowson, Penny and Williams (1961) found that potassium had only small and inconsistent effects upon the size of grain.

B. On quality

The factors defining quality in cereal grain vary, depending on how the grains are to be used. Most barley grain in Alberta is used for feeding livestock and poultry. A lesser proportion is used for direct human consumption, primarily in breakfast cereals, malt products, and beer. Only some of the general aspects of grain quality affected by fertilization, namely chemical composition which may influence the

nutritive value for livestock feed and malting quality for industrial use, will be reviewed.

(1) Chemical composition of grain

Man is interested in seed as a large source of nutrients and accessory nutrients for himself and other animals, and plant physiologists are interested in the materials stored in seeds as a source of nutrient material and energy for early development of the seedling. For malting purposes, the chemical composition is one of the criteria for estimating the commercial value of grain. The composition of any given crop of seeds may vary considerably from the average composition and two sets of factors determine this variation -- genetic and environmental. The latter includes the soil and climatic conditions. Disregarding the genetic and climatic factors, it was suggested that chemical composition of seed may be strongly affected by the nutrient supply in the soil on which the plants grow (Crocker and Barton, 1953, p. 22; Lavery, 1961, p. 10-11). This means that it may be possible to alter the composition of seed by controlling the fertility status of the soils. The change involves not only elements supplied by fertilizers but also other mineral elements or organic compounds found in the plant.

The crude protein ($N \times 6.25$) content may be of importance in determining the value of grain as livestock feed. In general, application of nitrogen fertilizer increases the protein content of grain when more of this nutrient is taken up than is utilized in the growth needs of the cereal plant (Atkins, et al., 1955; Bhatti, Bowland, Bentley and Zalik, 1963; Greaves and Pittman, 1946; Larter and Whitehouse, 1958; Leonard and Martin, 1963, p. 501-503; Martin and Mikkelsen, 1960; McBeath, 1959, p. 41; McBeath, Bentley, Lynch and Bowland, 1960; Reisenauer and Dickson, 1961;

Williams and Smith, 1954). Many workers have reported an increased protein content from grain to which nitrogenous fertilizers were applied in the late stages of growth (Beaven, 1947; Borowkowaki and Kozera, 1957; McBeath, 1959; Toogood, Bentley, Moore and Webster, 1962). Williams and Smith (1954), Martin and Mikkelsen (1960) reported that phosphorus tended to depress the protein content of wheat. Bentley, Gareau, Renner, and McElroy (1956), Kenwood (1947) and Renner, Bentley and McElroy (1953) have shown that sulfur-supplying fertilizers increased the protein content of hays grown on sulfur-deficient Grey Wooded soils.

It also has been suggested that fertilizer treatments may affect not only quantity but the quality of grain protein as well. Bentley, Carson and Bowland (1960) and Renner et al. (1953) have reported variations in the proportion of nine essential amino acids in wheat and barley as a result of fertilization and cropping sequence. The report revealed that an increase in quantity of protein was not always accompanied by an increase in protein quality.

Commercial fertilizers may be expected to influence malting quality especially when the applied materials cause change in the protein content of the grain (Frey and Robertson, 1953; Meredith et al., 1942). Atkins et al. (1955) quoted work by others that calcium and phosphorus application affected the malting quality adversely, while potassium lowered the protein content of the grain. Martin and Mikkelsen (1960) have also reported on the detrimental effect of nitrogen fertilization at higher rates on malting quality of barley.

Atkins et al. (1955) found that phosphorus application resulted in significant increases in phosphorus content of the grain, soluble protein in wort and percent soluble total malt protein, white Dubetz

and Wells (1965) showed that protein content was decreased by phosphorus fertilization.

McLeod (1965) showed that accumulation of total available carbohydrates (TAC) in alfalfa roots increased with rate of potassium and decreased with nitrogen fertilization; at high rates of nitrogen the carbohydrate content of orchardgrass stubble increased with potassium fertilization. He concluded that potassium fertilization facilitated the storage of carbohydrate reserves at high rates of nitrogen and that high rates of potassium without nitrogen fertilization were detrimental to the storage of carbohydrate reserves.

(2) Other agronomic characteristics

Nitrogen fertilization has shown significant effects on other agronomic characteristics such as heading date, plant height, and straw weight. For example, it was reported that the fertilizer treatments including nitrogen and phosphorus frequently result in earlier heading and maturity (McBeath, 1959, p. 42; Toogood et al., 1962).

II. Effects of Seed Size on Yield and Quality

It was emphasized by early workers that the part played by seed itself is exceedingly important in determining the yield and quality of the crops. The situation may be improved vastly by the use of a higher quality of seed, obtained by plant breeding and seed selection. Plant breeding attains no end without intelligent selection of the progeny. On the other hand, much of the work commonly called selection is practically a sorting of mixtures.

Carleton (1920, p. 184-185) stated that as early as 1906 Zavitz reported that in 37 of 40 separate tests large seed gave greater yields of grain and straw than did small seed. Carleton (1920, p. 185-186)

also quoted Cobb (1903) in New South Wales who showed that in addition to better yields generally resulting from the large seed, there was better germination of such seed, and the plants from large seed were more vigorous and produced grain of better quality. Kiesselbach (1924) in summarizing the results obtained by early workers, agreed with this principle when equal numbers of seed were used.

Recent results reported by the workers in Lacombe, Alberta, showed that large seeds produced more vigorous seedlings, more tillers and greater yields on the average than did small, medium, or bulk seed (Demirlacakmak et al., 1964; Kaufmann, 1958; Kaufmann and McFadden, 1960; Kaufmann and McFadden, 1963; McFadden, 1964). The effect of seed size on germination, emergence, early vegetative growth, and productivity of forage crops has also been studied by many workers (Beveridge and Wilsie, 1959; Black, 1959; Henson and Taynan, 1961; Stickler and Wasson, 1963). They concluded that early vegetative growth was proportional to seed size.

III. Non-genetic Occurrence of Loose Smut

Loose smut of barley caused by Ustilago nuda has been responsible for significant reductions in grain yield (Canada Department of Agriculture, 1957). A detrimental effect of U. nuda infection on malting quality was also reported by Metcalfe et al. (1963). Treatments are available that will control the disease, but these are generally impractical when large quantities of seed are involved. The main hope, however, in reducing losses from loose smut lies in the production of resistant varieties. Until resistant varieties are available in sufficient quantity to replace susceptible varieties at present in use, it would seem that any procedure that might result in reduced losses would be

worthy of consideration.

Taylor (1928) reported that small kernels of wheat carried three to five times as much loose smut as large kernels. Taylor and Harlan (1943) working with four varieties of barley, showed that the smaller, lateral kernels of six-rowed barley carried an average of 13.4 percent infection with loose smut compared with an average of 4.0 percent infection in the larger, central kernels. They also showed that separation into different sizes resulted in the infected embryos being concentrated in the smaller seeded lots. With two varieties under tests in two years they obtained an average infection of 1.8, 5.4 and 12.8 percent infection in large, medium and small seed respectively. These results were in agreement with the recent report of Demirlicakmak et al. (1963). It was noted by Fezer (1962) and McFadden, Kaufmann, Russell and Tyner (1960) that plots sown with large barley kernels produced fewer plants infected with loose smut than those seeded with small, medium or bulk seed.

Lukosevicius (1962) observed that the highest incidence of smut in inoculated plants occurred when they were grown under relatively high levels of nitrogen and low levels of potassium. He concluded that either the different nutrient levels to which the inoculated plants had been exposed had a predisposing influence on smut development or that some other factor, possibly nutrition, was influencing the infected progenies in the seedling stage. In a further study it was concluded that nutrition of infected seedlings is not an important factor in determining smut development, but that nutritional predisposition of the inoculated plant may be important (Lukosevicius, Klinck and Steppler, 1965).

IV. Chemical Composition of Seed in Relation to Biochemical Aspects of the Subsequent Growth

The barley grain in general is rich in carbohydrates, low in proteins and rather low in fats. The overall composition of barley is shown in Table 1.

Table 1. Overall Analyses of Barley (Cook, 1962)

<u>Substance analysed</u>	<u>Content (% dry weight)</u>
Starch	63 - 65
Sucrose	1 - 2
Reducing sugars	0.1 - 0.2
Other sugars	1
Soluble gums	1 - 1.5
Hemicellulose	8 - 10
Cellulose	4 - 5
Proteins (N x 6.25)	8 - 11
Lipids	2 - 3
Mineral matter	2
Other materials	5 - 6

The carbohydrates, fats, nitrogenous substances and minerals are known to serve as sources of energy and food for the development of the germinating embryo.

The chemical changes that occur during germination and subsequent growth are complex in nature. They consist of three main types; the breakdown of certain materials in the seed, the transport of materials from one part of the seed to another and especially from the endosperm to the embryo, and lastly the synthesis of new materials from the breakdown products formed (Mayer, Poljakoff-Mayber, 1963). Barnell (1937) reported that during the first 159 hours, twice as much of the dry weight translocated from the endosperm of barley to the embryo was used in growth as in respiration.

It was shown that fats could be hydrolyzed and then oxidized to supply energy needed for germination and basic units for fatty acid

resynthesis (Crocker and Barton, 1953, p. 175-179; Koller, Mayer, Poljakoff-Mayber and Klein, 1962, p.450).

Proteins are often broken down during germination with a concomitant rise in amino acids and amides, followed by protein synthesis in the growing part of the embryo (Bonner, 1950; Crocker and Barter, 1953, p. 33-39; Oota et al., 1953). Troughton (1962, p. 45) stated that the nitrogen content in a seed may affect the growth of very young seedlings in a similar manner to the way in which the level of nitrogen affects the growth of the older plant. Reid (1929a and 1929b) found that seeds having high nitrogen and low carbon contents produced seedlings with large amounts of shoot growth and small amounts of root growth, while seeds with low nitrogen and high carbon produced plants with small shoot and large root system. These findings indicated that the growth pattern of seedlings seemed to be more directly related to the nitrogen content of the seed than to the supply of nitrogen in the rooting media. Beaven (1947, p. 29) reported that seeds of higher nitrogen content produced a greater number of tillers than did seeds of lower nitrogen content.

Growth regulators may also be involved in germination. It was shown that the effects of gibberellic acid, and of the germinating embryo, on the endosperm were identical and thereby strengthened the argument for considering an endogenous gibberellin as the endosperm-mobilizing hormone which hastens the activity of hydrolytic enzymes (Paleg, 1960a and 1960b; Paleg, 1961; Paleg et al., 1962a, 1962b; Varner and Chandra, 1964; Varner, Chandra and Chrispeels, 1965). Stimulation of germination by gibberellic acid has also been found by Koller et al. (1962, p. 444-446). The possible significance has been increased by the detection of gibberellin-like substances in a number

of seeds (Phinney and West, 1960).

Crocker and Barton (1953, p. 52-54) stated that the concentrations of vitamins which serve as co-enzymes changed during germination.

The changes in the carbohydrates of barley during germination have been studied in detail, because of their importance in the malting process. The transport of carbohydrates from storage organs, namely endosperm to the growing part of the embryo has been shown in many cases (Fukui and Nikuni, 1956; Oota, Fujii and Osawa, 1953). Starch, sugars, and fructosan are considered to be the available carbohydrates for respiration and growth of seedlings. Koller et al. (1962, p. 451-452) divided the sugars appearing during germination into two groups: those that are found to be closely connected with respiration, i.e., raffinose, sucrose, and glucodifuctose as well as fructosan; and those that are found to be the results of starch breakdown, i.e., glucose, fructose, maltose, etc.

It was suggested in the previous part of the literature review that large seed produced more vigorous seedling than did small seed. It may be postulated that the superiority of large grains result from their containing more storage materials so that they are able to provide the young plant with more assistance in its early struggle for life than can a grain of much less weight. The quantitatively most important components of grain are available carbohydrates and proteins. It was thought that determination of the proportions of these, in seeds of different sizes and seeds from variously fertilized parents, might contribute to an explanation of the difference in performance of those seeds.

V. Summary of the Reviewed Literature

1. Suitable fertilization increases the yield and improves the quality of cereal crops.

2. Seed size has shown significant effects on early growth and final yield.

3. The early growth of plants may depend on the quantity or quality of the reserve materials in grain.

MATERIALS AND METHODS

I. Materials

Seeds obtained from five field fertilizer experimental locations were used in the course of the study. Information about the sources of seeds is given in Table 2.

The 1964 sites for the production plots were chosen by selecting soils which contained low available nitrogen and phosphorus shown by the soil test data, while the 1963 plots were part of another study. All sites were located in the Black, and Dark Grey Wooded soil zones of Alberta.

II. Methods

A. Preparation of barley seed

After broken kernels and weed seeds were removed by hand picking, each cleaned seed sample was separated into three size categories, small, medium and large, by sieve separation. A fanning mill (sieve and air-blast cleaner) with two different sizes of sieves was used as seed separator. Sieves used for separating the seed samples of sites 1, 3, 4, 5 were $6\frac{1}{2}/64'' \times 3/4''$ and $6/64'' \times 3/4''$, while for site 2 they were $7/64'' \times 3/4''$ and $6/64'' \times 3/4''$. The sample was put over the sieves 10 - 15 times. Those seeds that passed through a sieve $6/64'' \times 3/4''$ were designated "small"; while seeds that passed over a sieve $6\frac{1}{2}/64'' \times 3/4''$ or $7/64'' \times 3/4''$ were designated "large".

B. Laboratory evaluation of barley seed

(1) Size fraction and percent germination

The fractions obtained were weighed and the percent of each was calculated. 1000 kernel weight of each size of seed was determined by

Table 2. Some Information Concerning the Seed Production
and the Seed Evaluation Plots

Seed Production Plots			Seed Evaluation Plots								
Location No.	Name of Cooperator	Year seed produced	Available nutrients (a) (lb/acre)			Barley yields (cwt/acre) (d)				Site	Year
			N	P	K	N fert. (b)		P fert. (c)			
						0	+	0	+		
1	H. Evjen	1963	16	161	130	7.3	15.6*	11.6	11.3	Field	1964
2	H. Schlecker	1963	20	60	116	18.7	26.1*	20.9	23.9	Greenhouse	1965
3	G. Neuman	1964	16	4	30	9.3	19.3**	12.5	16.1**	Field	1965
4	J. Perrin	1964	16	20	42	20.2	25.1**	22.0	23.4*	Field	1965
5	N. Byer	1964	16	42	50	10.8	22.2**	16.6	16.5	Field	1965

(a) Available nutrients determined at time of seeding on 0 - 6" composite samples

(b) Nitrogen fertilizer rates at Sites 1 and 2 ... 0 and 80 lbs/acre; at Sites 3 - 5 ... 0 and 60 lbs/acre

(c) Phosphorus fertilizer rates at Sites 1 and 2 ... 0 and 34 lbs/acre; at Sites 3 - 5 ... 0 and 48 lbs/acre

(d) ** Statistical significance at 1% level; * at 5% level

1. The first part of the assignment is to find the value of the function $f(x)$ at $x = 1$.

2. The second part is to find the value of the function $f(x)$ at $x = 2$.

3. The third part is to find the value of the function $f(x)$ at $x = 3$.

4. The fourth part is to find the value of the function $f(x)$ at $x = 4$.

5. The fifth part is to find the value of the function $f(x)$ at $x = 5$.

6. The sixth part is to find the value of the function $f(x)$ at $x = 6$.

7. The seventh part is to find the value of the function $f(x)$ at $x = 7$.

8. The eighth part is to find the value of the function $f(x)$ at $x = 8$.

9. The ninth part is to find the value of the function $f(x)$ at $x = 9$.

10. The tenth part is to find the value of the function $f(x)$ at $x = 10$.

11. The eleventh part is to find the value of the function $f(x)$ at $x = 11$.

12. The twelfth part is to find the value of the function $f(x)$ at $x = 12$.

13. The thirteenth part is to find the value of the function $f(x)$ at $x = 13$.

14. The fourteenth part is to find the value of the function $f(x)$ at $x = 14$.

15. The fifteenth part is to find the value of the function $f(x)$ at $x = 15$.

16. The sixteenth part is to find the value of the function $f(x)$ at $x = 16$.

17. The seventeenth part is to find the value of the function $f(x)$ at $x = 17$.

18. The eighteenth part is to find the value of the function $f(x)$ at $x = 18$.

19. The nineteenth part is to find the value of the function $f(x)$ at $x = 19$.

20. The twentieth part is to find the value of the function $f(x)$ at $x = 20$.

weighing out duplicate, approximately 20 gram sample, and counting the number of kernels in each with an electronic seed counter. The determination of percent germination of seed was done by Plant Products Division, Canada Department of Agriculture in Edmonton.

(2) Chemical Analyses

The seed was analyzed for Kjeldahl nitrogen, free sugars (80% alcohol extractable sugars) and residual available carbohydrates.

Kjeldahl nitrogen analysis

Samples were ground to pass through a 20-mesh sieve with a Wiley mill. The ground samples were stored in glass bottles and dried at 70°C overnight prior to the determination. Total reduced nitrogen content of seed was determined by the improved Kjeldahl method with the following modifications: (a) a commercial Kel-Pak catalyst was used rather than a salt mixture for digestion, and (b) distillation was done into boric acid solution containing the mixed indicator brome cresol green and methyl red followed by titration with standard sulphuric acid. The values were expressed as percent on the dry weight basis (A.O.A.C., 1960).

Free sugar and residual available carbohydrate analyses

The samples were prepared for analysis by grinding in a Wiley mill using a 40-mesh sieve. The prepared samples were then stored in the glass bottles and dried at 70°C overnight prior to analysis. The quantitative determinations of free sugars and residual available carbohydrates were done following a combination of methods that were described by Clegg (1956) and Smith, Paulsen and Raguse (1964).

The finely ground sample was extracted three times with hot 80% ethanol. Since ethanol interferes with color development in the anthrone-sugar reaction, it was removed from the combined extracts by evaporation on a boiling water bath. The remaining aqueous fraction

was diluted with water. The diluted solution was then ready for free sugar analysis.

After the free sugars were removed from the sample, the residue was hydrolyzed with 50 ml. of 0.2N sulphuric acid for an hour in a boiling water bath. Hydrolysates were diluted with water.

The concentrations of free sugars in the diluted, alcohol-free extracts and residual available carbohydrates in the diluted acid hydrolysates were determined by anthrone-sulphuric acid colorimetric method (Clegg, 1956) using glucose to prepare the standard curve. The values of both free sugars and residual available carbohydrates were expressed as the equivalent quantity of glucose, calculated as percent on the dry weight basis.

C. Greenhouse evaluation of barley seeds

Seeds from one site were evaluated in a greenhouse trial conducted during the late winter and early spring of 1965. The soil on the greenhouse bench was fertilized. Phosphorus was applied as superphosphate at the rate of 1 pound per 100 sq. feet and potassium as potassium chloride at the rate of 3/4 pound per sq. feet. Both were evenly broadcast over the bench just a day before seeding. No nitrogen fertilizer was applied at seeding time, but it was added as ammonium nitrate twice during the early growing period when soil test data indicated a need. Small, large, and cut large[#] seeds from parents which received different fertilizers, were sown carefully at a uniform depth of 1½ inches in staggered rows so that the interplant distance was 6 inches in all directions. The randomized design with three replicates was arranged on the bench. Temperature in the compartment was controlled at 68°F, artificial light was on from

[#] The cut large seed was prepared by removing part of the endosperm of a large seed with a sharp knife prior to seeding.

7:00 a.m. to 11:00 p.m. throughout the experimental period. Liquid insecticide (Diazinon, 5 c.c./gal.) was sprayed twice in growing season.

One plant at each end of the row was considered as a border plant and measurements were not made on it. Plant growth was evaluated by observing or measuring the following: (1) time to emergence; (2) time for appearance of first few leaves; (3) width of first leaf at two leaf stage; (4)[#] plant height at several stages; (5) time to heading; (6) number of healthy heads at harvest; and (7) grain and straw yields at harvest.

D. Field evaluation of barley seeds

Field trials to evaluate the seed were conducted on the University Farm at Ellerslie during the summers of 1964 and 1965. Four seed sizes (small, medium, large and bulk) harvested from parents on each of four differently fertilized plots were seeded in the 1964 field trial in a randomized complete block design replicated four times. Plots consisted of four rows, 15 feet long, with 9 inch spacing between rows and plots. Germination and 1000 kernel weight data were used to calculate the weight of seed to be planted in each row.

In 1965 two seed sizes (small and large) harvested from parents on each of four differently fertilized plots were seeded. The same field design as in 1964 field trials was used.

In both years, information was obtained from the two central rows of each plot. Seedlings were counted at the two-leaf-stage on the area to be harvested in 1964 plots only. Seedling vigor was evaluated at the four to five-leaf-stage. In the fall the two central rows, 12 feet long, were cut by hand. The total number of heads was counted and the percent smutted heads was determined. After threshing, grain and

[#] Measurements of plant hieght were made during the growing period, but were not made during the period after awns started to emerge until harvest time.

straw yields were measured and the 1000 kernel weight of bulked seed harvested from each plot was determined.

E. Statistical analyses:

Analyses of variance were carried out on the results from laboratory studies, greenhouse and field trials; the data were analyzed as a factorial design. This was done on an IBM computer using Library Program "BMD02V" which was written by the Health Sciences Computing Facility, Department of Preventative Medicine and Public Health, School of Medicine, University of California, Los Angeles, California. The computer program printed out (a) mean square values from which the appropriate F-Ratios were calculated as shown in Table 3, and (b) mean values for all levels of each factor averaged across all other factors. It was these average values which were recorded in the following tables.

Table 3. Analyses of Variance

Source of Variance	No. of Levels	Degrees of Freedom	Expected Value of Mean Square
1 Replication (R)	r	r - 1	$\sigma_e^2 + \text{snp } \sigma_R^2$
2 Seed size (S)	s	s - 1	$\sigma_e^2 + \text{np } \sigma_{RS}^2 + \text{rnp } \sigma_S^2$
3 Nitrogen (N)	n	n - 1	$\sigma_e^2 + \text{sp } \sigma_{RN}^2 + \text{rsp } \sigma_N^2$
4 Phosphorus (P)	p	p - 1	$\sigma_e^2 + \text{sn } \sigma_{RP}^2 + \text{rsn } \sigma_P^2$
12 R x S		(r - 1)(s - 1)	$\sigma_e^2 + \text{np } \sigma_{RS}^2$
13 R x N		(r - 1)(n - 1)	$\sigma_e^2 + \text{sp } \sigma_{RN}^2$
14 R x P		(r - 1)(p - 1)	$\sigma_e^2 + \text{sn } \sigma_{RP}^2$
23 S x N		(s - 1)(n - 1)	$\sigma_e^2 + \text{p } \sigma_{RSN}^2 + \text{rp } \sigma_{SN}^2$
24 S x P		(s - 1)(p - 1)	$\sigma_e^2 + \text{r } \sigma_{RSP}^2 + \text{rn } \sigma_{SP}^2$
34 N x P		(n - 1)(p - 1)	$\sigma_e^2 + \text{s } \sigma_{RNP}^2 + \text{rs } \sigma_{NP}^2$
123 R x S x N		(r - 1)(s - 1)(n - 1)	$\sigma_e^2 + \text{p } \sigma_{RSN}^2$
124 R x S x P		(r - 1)(s - 1)(p - 1)	$\sigma_e^2 + \text{n } \sigma_{RSP}^2$
134 R x N x P		(r - 1)(n - 1)(p - 1)	$\sigma_e^2 + \text{s } \sigma_{RNP}^2$
234 S x N x P		(s - 1)(n - 1)(p - 1)	$\sigma_e^2 + \sigma_{RSNP}^2 + \text{r } \sigma_{SNP}^2$
1234 R x S x N x P		(r - 1)(s - 1)(n - 1)(p - 1)	$\sigma_e^2 + \sigma_{RSNP}^2$
E			σ_e^2

F - Ratio for testing significance: Size = 2/12; N = 3/13; P = 4/14; SxN = 23/123; SxP = 24/124;
Nxp = 34/134

RESULTS AND DISCUSSION

I. Germination, Percent Size Fraction, and 1000 Kernel Weight of Seeds Evaluated in the Field and Greenhouse

Percent germination (Table 4), was determined in order to adjust the quantity of seed planted per row. Although the percent germination differed between locations, the percent germination of large and small seeds from each source was the same. Further, there was no effect of parental fertilization on percent germination.

The proportion of large seed was significantly increased and that of small seed was decreased by nitrogen fertilization at all locations except number 2 (Table 5). Phosphorus fertilization showed the same trend at three locations, but none of the differences was significant at the 5% level.

Nitrogen fertilization increased the average weight of kernels at three locations but decreased it, though not significantly, at location 5 (Table 6). Similar results were obtained from phosphorus fertilization. A strong S x N interaction at location 3 and S x P and N x P interactions at location 5 were noted. The significant S x N interaction at location 3 indicated that nitrogen fertilization increased the 1000 kernel weight of large seed in every case, but either decreased that of small seed or increased it to a lesser extent. The S x P interaction at location 5 indicated that the degree of phosphorus fertilizer effect shown on large seed was greater than on small seed. The N x P interaction indicated that 1000 kernel weight of seed was significantly decreased when nitrogen was applied without any supply of phosphorus, and was increased when nitrogen was applied along with phosphorus; and vice versa.

Table 4. Percent Germination of Seeds Evaluated in the Field and Greenhouse

		Seed from Location				
		1	2	3	4	5
Seed Size (S)	Small	94	93	80	88	88
	Medium	96	-	-	-	-
	Large	97	90	82	87	88
	Bulk	96	-	-	-	-
Parental Fertilization (b)	Nitrogen (N)	0	96	93	79	88
	+	96	89	83	87	90
	Phosphorus (P)	0	95	93	83	88
	+	96	90	79	87	87

(a) No statistical analysis was done

(b) 0 = No Nitrogen or Phosphorus fertilization

+ = Nitrogen or Phosphorus fertilization

Table 5. Percent Size Fraction of Seeds^(a) Evaluated in the Field and Greenhouse

	Seed from Location										
	1 (b)		2 (b)		3		4		5		
	% Small	% Large	% Small	% Large	% Small	% Large	% Small	% Large	% Small	% Large	
Fertilization											
	0	31.5	31.1	9.7	34.2	34.1	38.3	13.6	51.8	31.7	35.2
Nitrogen (N)	+	13.6	45.1	15.7	19.5	25.6*	43.7†	8.4*	60.2*	12.5**	49.7*
Phosphorus (P)	0	20.0	40.0	14.6	32.1	31.8	39.2	12.0	54.3	23.5	41.3
	+	21.2	38.5	11.8	21.6	27.9†	42.9†	10.0	57.8	20.8	43.6
N x P interaction	-	-	-	-	-	ns	ns	ns	ns	ns	ns

(a) On weight basis

(b) Statistical analysis could not be done on the data from locations 1 and 2, because the samples from these two locations were bulked samples of six replicates

** Statistical significance at 1% level

* " " 5% level

† " " 10% level

ns Not significant

Table 6. 1000 Kernel Weight of Seeds Evaluated in the Field and Greenhouse

Seed Size (S)	Seed from Location				
	1(b)	2(b)	3	4	5
Small	25.8	24.5	22.0	25.7	24.9
Medium	33.7	-	-	-	-
Large	41.9	47.1	38.4**	41.3**	40.4**
Bulk	34.1	-	-	-	-
Fertilization					
Nitrogen (N)	0	32.0	35.8	29.7	33.1
	+	35.2	35.9	30.6†	33.9*
Phosphorus (P)	0	33.8	35.8	29.6	32.8
	+	34.0	35.9	30.7*	34.2**
Interaction	S x N	-	-	**	ns
	S x P	-	-	ns	**
	N x P	-	-	ns	**

(a) 1000 kernel weight in g

(b) No statistical analysis was done on these two locations because the samples from these locations were bulked samples of six replicates

** Statistical significance at 1% level

* " " 5% "

† " " 10% "

ns Not significant

II. Greenhouse Evaluation

The time to emergence was virtually the same for both small and large seeds from unfertilized and nitrogen or phosphorus fertilized parents. The cut large seed showed a slight delay in emergence. Although time of emergence was virtually the same, plants grown from large seeds were considerably more vigorous than plants grown from small seeds, and much more vigorous than plants from cut large seeds. Measurements of plant height were made during the growing period until awns started to emerge and then at harvest time (Table 7 and Fig. 1). The width of first leaf at the two leaf stage was recorded (Table 8). Significant effects of seed size on plant height prior to heading and on leaf width were noted. Observation indicated that the time for emergence of the first few leaves was 2 - 4 days less for plants grown from large seeds than for plants grown from small and cut large seeds. These differences due to seed size were especially obvious at early growth stages, and gradually disappeared until awns started to emerge. By that time no visible difference could be observed. There was a tendency for parental nitrogen fertilization to increase the plant height (Table 7 and Fig. 2) and width of leaf during the early growth period, while parental phosphorus fertilization showed no influence on early growth (Tables 7 and 8). The S x N, S x P, and N x P interactions were insignificant.

The plants grown from large seeds started heading earlier than those from small seeds and much earlier than those from cut large seeds (Table 8). Parental nitrogen or phosphorus fertilization showed no effect on time to heading.

The number of healthy heads per plant grown from small and large seed was not significantly different but cut large seed produced fewer

Table 7. Plant Heights Evaluated in the Greenhouse (a)

		Age of Plant when measurement made (days)						
		8	15	20	24	28	33	37 at harvest
Seed Size (S)	Small	7.5	17.8	31.5	39.2	48.7	61.8	118.6
	Large	8.7*	21.7*	34.8*	45.6**	52.5*	64.4*	119.6
	Cut ^(b)	5.5	13.0	24.0	33.0	43.0	56.0	116.5
Parental	Nitrogen (N)	0	7.9	19.3	32.7	41.8	49.8	73.3
		+	8.3†	20.2**	33.6	43.0*	51.0	74.3
Fertilization	Phosphorus (P)	0	8.0	19.7	33.3	42.5	50.5	73.8
		+	8.2	19.8	32.9	42.3	50.3	73.7
Interaction	S x N	ns	ns	ns	ns	ns	ns	ns
	S x P	ns	ns	ns	ns	ns	ns	ns
	N x P	ns	ns	ns	ns	ns	ns	ns

(a) Plant height in cm

(b) Data from cut large seed were not included in statistical analysis

** Statistical significance at 1% level

* " " 5% "

† " " 10% "

ns Not significant

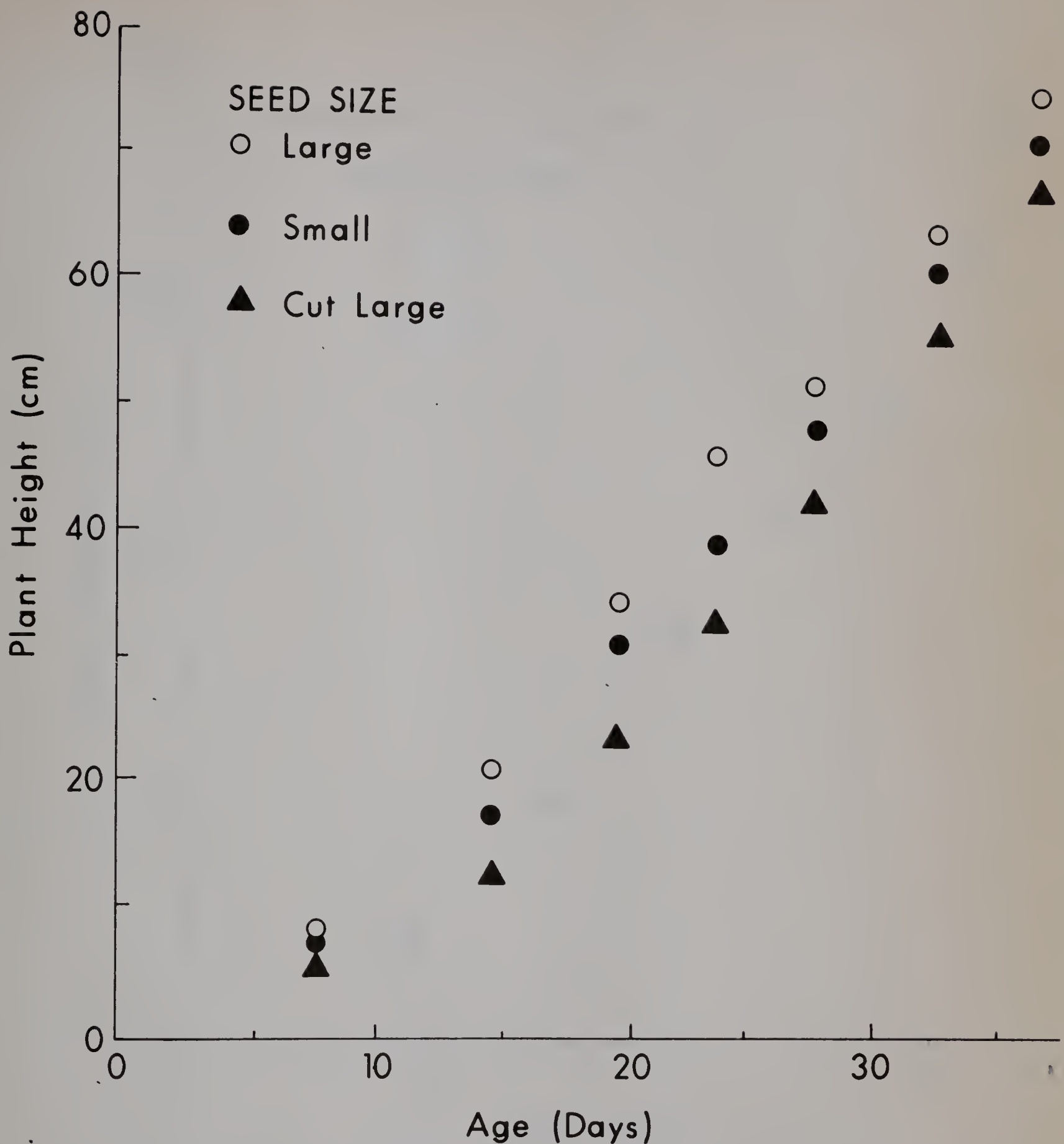


FIG 1 Heights, at various ages, of plants grown from small and large seeds in the greenhouse.

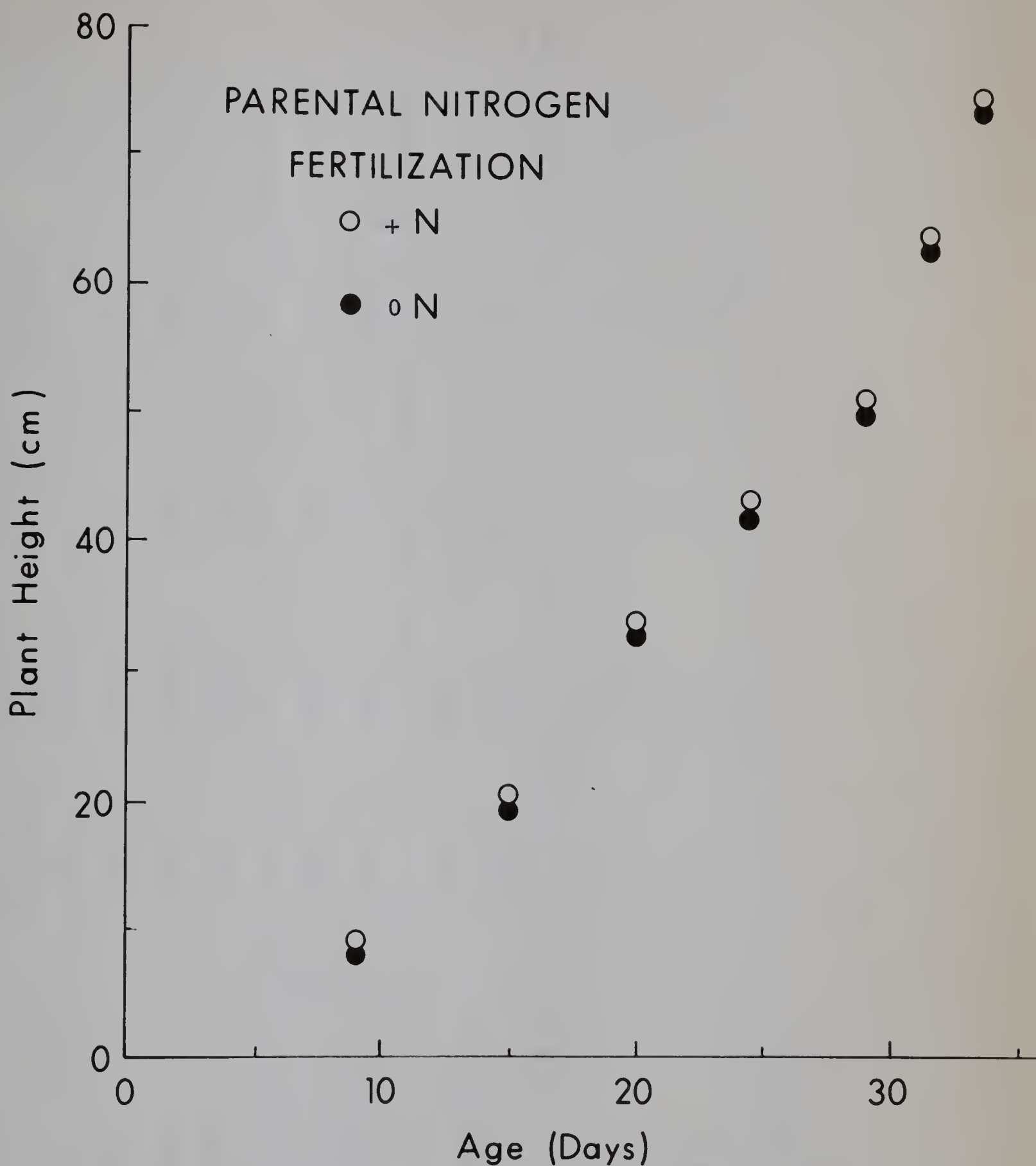


FIG 2 Heights of plants, grown with seeds from nitrogen fertilized and unfertilized parents, in the greenhouse.

Table 8. Measurements of Other Agronomic Characteristics of Plants in the Greenhouse

		Width of first leaf (cm)	Time to heading (day)	No. healthy heads/plant	Grain yield (g/plant)	Straw yield (g/plant)
Seed Size (S)	Small	0.78	43.5	6.0	7.8	11.0
	Large	0.98*	42.1*	6.3	8.9	11.3
	Cut (a)	0.70	48.0	5.0	6.7	8.5
Parental	Nitrogen (N)	0	42.8	6.0	8.3	10.9
		+	42.8	6.3	8.4	11.5
Fertilization	Phosphorus (P)	0	42.7	6.3	8.3	11.0
		+	42.9	6.1	8.4	11.4
Interaction	S x N	ns	ns	ns	ns	ns
	S x P	ns	ns	ns	ns	ns
	N x P	ns	ns	ns	ns	ns

(a) Data from cut large seed were not included in statistical analysis

* Statistical significance at 5% level

ns Not significant

healthy heads (Table 8). There was no effect of parental nitrogen or phosphorus fertilization on number of healthy heads per plant. The S x N, S x P, and N x P interactions were insignificant.

Large seeds tended to produce greater grain and straw yields than did small seeds, and cut large seeds (Table 8), but these differences were not significant. There was no effect of parental fertilization on grain yields but just a slight, but non-significant, trend that, for a given seed size, seed from nitrogen fertilized parents produced higher straw yields than seed from unfertilized parents. Similarly, seed from phosphorus fertilized parents tended to produce more straw than did that from unfertilized parents. The S x N, S x P, and N x P interactions were not significant.

III. Field Evaluation

Emergence of seedlings was counted on the 1964 plots only (Table 9). The same number of seedlings were expected to emerge on each plot because seeding rate was based on germination data, but large seeds produced more seedlings than did small seeds. Further, a significant increase in seedling count was observed with seeds from parents which had received nitrogen fertilizer; no effect of parental phosphorus fertilization on emergence was noted. A significant S x N interaction indicated that the effect of parental nitrogen fertilization on emergence was not the same for all seed size categories.

There was a striking similarity between early growth stages in all the field trials and the greenhouse studies. Marked differences in early growth associated with seed size were noted. Again, plots sown to large seeds and small seeds were easily distinguished shortly after emergence when the plants from large seeds appeared much more vigorous. The more

Table 9. Emergence of Seedlings in the Field
using Seeds from Location 1 (a)

Seed Size (S)	Small	359
	Medium	368
	Large	376*
	Bulk	372
Fertilization	Nitrogen (N)	0
		+
	Phosphorus (P)	0
		+
Interaction	S x N	*
	S x P	ns
	N x P	ns

(a) Number of seedlings per plot
 ** Statistical significance at 1% level
 * " " 5% level
 ns Not significant

rapid growth rate of plants from large seeds was manifested in earlier development of second and subsequent leaves. The early growth evaluation observed in the 1964 field trial showed that the behaviors of medium and bulk seed were the same.

The total number of healthy heads per plot from large seeds was greater than that from small seeds in all cases, and four out of five cases were statistically significant (Table 10). When the above results were calculated as number of healthy heads per plant, the same trend was observed. Parental nitrogen or phosphorus fertilization showed irregular effects on both total number of healthy heads per unit area or per plant.

The differences in grain yield due to seed size were statistically significant in four out of five cases, and this exceptional case showed the same trend (Table 11). Again, large seed produced heavier grain than did small seed (Table 12). No effect of parental nitrogen or phosphorus fertilization on grain yield and little or no effect on 1000 kernel weight was found.

Similar results were shown in straw yield (Table 13) as were found in grain yields. Straw yield per plot was significantly greater in plots sown with large seeds than from small seeds. No consistent effect of parental nitrogen on straw yield was found, while in four out of five cases parental phosphorus fertilization tended to decrease straw yield, but none of them was significant. Significant S x N interactions were found in three out of five cases, indicating that parental nitrogen fertilization affected the straw yield produced from small and large seed to various extents.

Because it has been reported by many workers that small and medium seeds carry more loose smut than large seeds in composite samples of

Table 10. Total Number of Healthy Heads per Plot on Seed Evaluation Plots

	Seed from Location				
	1	2	3	4	5
Seed Size (S)					
Small	481	423	396	466	436
Medium	492	-	-	-	-
Large	504	475*	448*	481*	467*
Bulk	485	-	-	-	-
Parental					
Fertilization	0	430	437	473	459**
	+	467	406	474	444
Phosphorus (P)	0	457	440**	472	461
	+	440	404	475	442
S x N	ns	ns	†	ns	ns
S x P	ns	ns	ns	ns	ns
N x P	ns	ns	ns	*	†

** Statistical significance at 1%
 * " " 5%
 † " " 10%
 ns Not significant

Table 11. Grain Yields Produced on Seed Evaluation Plots^(a)

	Seed from Location				
	1	2	3	4	5
Seed Size (S)					
Small	492	433	414	460	431
Medium	497	-	-	-	-
Large	511	519**	486*	505†	515*
Bulk	499	-	-	-	-
Parental					
Nitrogen (N)	0	506	454	453	487
	+	494	499	448	478
Fertilization					
Phosphorus (P)	0	499	481	463	471
	+	501	471	438	494
Interaction					
S x N	ns	ns	†	ns	ns
S x P	ns	ns	ns	ns	ns
N x P	ns	ns	ns	*	ns

(a) Grain yields given in g per plot
 ** Statistical significance at 1% level
 * " " 5%
 † " " 10%
 ns Not significant

Table 12. 1000 Kernel Weight of Bulk Seeds Harvested from the Seed Evaluation Plots^(a)

		Seed from Location					
		1	2	3	4	5	
Seed Size (S)	Small	35.9	30.4	30.1	31.4	31.9	
	Medium	36.6	-	-	-	-	
	Large	36.4	30.9†	30.9	32.3	32.7	
	Bulk	35.9	-	-	-	-	
Parental	Nitrogen (N)	0	36.3	30.7	30.5	31.8	31.9
		+	36.1	30.6	30.5	31.8	32.7*
Fertilization	Phosphorus (P)	0	35.9	30.6	30.3	31.5	32.2
		+	36.5†	30.7	30.7	32.2†	32.3
Interaction	N x S	†	ns	ns	ns	ns	ns
	S x P	†	ns	ns	ns	ns	ns
	N x P	ns	ns	ns	ns	ns	ns

(a) 1000 kernel weight in g
 † Statistical significance at 10% level
 ns Not significant

Table 13. Straw Yields Produced on Seed Evaluation Plots^(a)

Seed Size (S)	Seed from location				
	1	2	3	4	5
Small	236	415	381	450	430
Medium	285	-	-	-	-
Large	297†	488**	471*	502**	517†
Bulk	266	-	-	-	-
<hr/>					
Parental	Nitrogen (N)				
	0	268	431	446†	483
Fertilization	Phosphorus (P)				
	0	277	465	436	473
Interaction	S x N				
	+	265	437	416	479
Interaction	S x P				
	+	265	437	416	479
Interaction	N x P				
	+	265	437	416	479
<hr/>					
Interaction	S x N				
	+	265	437	416	479
Interaction	S x P				
	+	265	437	416	479
Interaction	N x P				
	+	265	437	416	479

(a) Straw yields given in g per plot
 ** Statistical significance at 1% level
 * " " 5%
 † " " 10%
 ns Not significant

barley, and because Lukosevicius et al. (1965) concluded that nutrition of infected seedlings is not an important factor in determining smut development, but that nutritional predisposition of the inoculated plant may be important, the occurrence of smutted heads in the field was recorded at harvest time. Plots sown to large seeds showed a lower incidence of loose smut than did those sown to small seeds (Table 14). The variation in percent smutted heads due to seed size was statistically significant in four out of five cases, and the fifth case also showed the same trend. Significant S x N interactions in locations 1 and 2, and N x P interactions in locations 3 and 4 were noted. These significant interactions can be interpreted as stated previously. A significant effect of parental nitrogen fertilization on percent smutted heads was observed in the plots grown from seed from location 1. Seeds from parents which received nitrogen fertilizer produced plants with more smutted heads than did seeds from unfertilized parents. In the other seed evaluation plots there was no consistent effect of parental fertilization on the occurrence of loose smut. The interesting result found in plots grown with seeds from locations 1 and 2 was that the relation between parental nitrogen fertilization and susceptibility to loose smut obviously varied with seed size. Small seed produced plants with more or less the same percent smutted heads, no matter whether those small seeds were from nitrogen fertilized or unfertilized parents; while large seeds from nitrogen fertilized parents produced many more smutted heads than did those from unfertilized parents. The latter may be explained as follows: since under nitrogen fertilization more lateral kernels are large enough to remain on the "large" separation sieve and it is mostly the laterals which carry loose smut,

Table 14. Percent Smutted Heads Produced on Seed Evaluation Plots

	Seed from Location				
	1	2	3	4	5
Seed Size (S)					
Small	5.2**	5.7*	2.0*	1.7†	2.2
Medium	3.9	-	-	-	-
Large	1.7	2.5	1.0	0.8	1.6
Bulk	3.1	-	-	-	-
Parental					
Nitrogen (N)	0	2.6	4.4	1.6	1.2
	+	4.4*	3.9	1.4	1.2
Fertilization					
Phosphorus (P)	0	3.8	4.2	1.0	1.1
	+	3.2	4.1	1.9*	1.4
Interaction					
S x N	*	*	ns	ns	ns
S x P	ns	ns	ns	ns	ns
N x P	ns	ns	**	†	ns
** Statistical significance at 1% level					
* " " 5%					
† " " 10%					
ns Not significant					

this may account for the increase in the incidence of loose smut in plants grown from large seeds in this particular instance.

IV. Chemical Composition of Seeds Evaluated in the Field and Greenhouse

Percent Kjeldahl nitrogen and percent total available carbohydrates including free sugars and residual available carbohydrates (RAC) were determined. There was not much difference in percent Kjeldahl nitrogen (Table 15) between small and large seeds, although there was a tendency that percent Kjeldahl nitrogen of large seed was slightly higher than that of small seed. As was expected nitrogen fertilization significantly increased the percent nitrogen content in the seed. On the contrary phosphorus fertilization tended to cause a decrease in percent Kjeldahl nitrogen in all cases except seeds from location 1.

The percent free sugars of large seed was considerably higher than that of small seed (Table 16). The increase in percent free sugars due to nitrogen fertilization was found statistically significant. No consistent effect of phosphorus fertilization was observed. Significant S x P, N x P interactions in location 2, and S x N, N x P interactions in location 4 were found. Again the N x P interaction indicated that application of phosphorus without nitrogen decreased the percent free sugars. The same interpretation as stated previously can also be applied to explain the significant S x P, S x N interactions found here.

The percent RAC of large seed was higher than that of small seed in three out of five cases, and lower in two cases (Table 17). There was an apparent decrease in percent RAC due to nitrogen fertilization. In three out of five cases phosphorus fertilization tended slightly to increase percent RAC. Strong S x N, S x P, and N x P interactions were found in seeds from location 2. The S x N interaction indicated that the

Table 15. Percent Kjeldahl Nitrogen of Seeds^(a) Evaluated in the Field and Greenhouse

	Seed from Location				
	1	2	3	4	5
Seed Size	2.11	2.20	1.62	1.95	1.80
	2.16	-	-	-	-
	2.12	2.23	1.73	2.10	1.91
	2.12	-	-	-	-
Fertilization	0	1.77	1.93	1.57	1.80
	+	2.37	2.50	1.79	2.26
	0	2.11	2.25	1.75	2.06
	+	2.14	2.17	1.61	2.02

(a) On dry weight basis

No statistical analysis was done on these data because an unequal number of determinations were done on samples from various treatments.

Table 16. Percent Free Sugars of Seeds^(a) Evaluated in the Field and Greenhouse

	Seed from Location				
	1 (b)	2	3	4	5
Seed Size (S)					
Small	4.3	3.5	2.8	3.4	3.1
Large	5.1	4.2**	2.8	4.3**	3.5**
Fertilization					
Nitrogen (N)	0	3.8	3.5	2.7	3.6
	+	5.6	4.2**	2.9*	4.1**
Phosphorus (P)	0	4.8	4.0	2.8	3.8
	+	4.6	3.8	2.8	3.9
Interaction					
S x N	-	ns	ns	**	ns
S x P	-	**	ns	ns	ns
N x P	-	*	ns	**	ns

(a) On dry weight basis

(b) No statistical analysis was done because an unequal number of determinations were done on samples from various treatments

** Statistical significance at 1% level

* " " 5% "

ns Not significant

Table 17. Percent Residual Available Carbohydrates of Seeds^(a)
Evaluated in the Field and Greenhouse

		Seed from Location				
		1 (b)	2	3	4 (b)	5
Seed Size (S)	Small	64.8	65.0	70.4	71.5	71.6†
	Large	66.5	67.2*	73.2*	70.6	71.0
Fertilization	Nitrogen (N)	0	67.8*	73.0†	73.1	73.3**
		+	64.4	70.6	68.9	69.4
	Phosphorus (P)	0	65.7	70.9	70.8	71.6
		+	65.6	72.7†	71.3	71.0
Interaction	S x N	-	**	ns	-	ns
	S x P	-	**	ns	-	ns
	N x P	-	*	ns	-	ns

(a) On dry weight basis
 (b) No statistical analysis was done because an unequal number of determinations were done on samples from various treatments
 ** Statistical significance at 1% level
 * " " 5%
 † " " 10%
 ns Not significant

degree of a decrease in percent RAC due to nitrogen fertilization was greater on small seed than on large seed, whereas S x P interaction indicated that the degree of phosphorus fertilizer effect shown on large seed was greater than that on small seed. The N x P interaction indicated that when nitrogen was applied, phosphorus fertilization increased percent RAC; when nitrogen was absent, phosphorus fertilization either decreased percent RAC or increased it to a lesser extent than when nitrogen was present.

GENERAL DISCUSSION

I. Effects of Seed Size on Early Plant Growth and Yields

The insignificant difference in number of healthy heads per plant grown in the greenhouse from small and large seed might be due to the reduction in competition between plants, and suggests that tillering capacity might be the same for both small and large seeds, if the competition between plants is prevented. In the field studies the number of healthy heads per plant grown from large seed was relatively greater than for plants from small seed, and in most of the cases were statistically significant. These results are in agreement with the report of Kaufmann and McFadden (1960) that increased competition favoured plants from large seeds. The result that plants produced from large seed had many fewer smutted heads than those from small seed, agrees with the recent report by Demirlicakmak and Kaufmann (1963) and McFadden et al. (1960).

The previous results reported for both greenhouse and the field studies indicated that for a given number of seeds, large seeds produced more vigorous seedlings, a greater number of healthy heads, and greater grain and straw yields than did small seeds. Further work might be needed to show, for a given weight of seeds, if there would be any difference in final yields between plots sown to small and large seeds.

There are several possible explanations of the previous results that the behaviour both in early growth and final yields of large seed was superior to that of small seed. One possibility is that large seed might produce a greater root system so that the absorption of external nutrient is greater than that of small seed. Another possibility may be the differences in the nature of the materials stored in the seed. The

latter possibility was investigated. Since proteins and available carbohydrates are the quantitatively significant components in barley seed, results of percent Kjeldahl nitrogen and percent available carbohydrates including free sugars and residual available carbohydrates were reported in the previous section.

Because the percent total available carbohydrates (TAC) of large seed was more or less the same as that of small seed while the average kernel weight of large seed was considerably heavier than that of small seed, it is obvious that the total amount of available carbohydrates of large seed is much greater than that of small seed as shown in Table 18. The same result was found in total amount of nitrogen in seed of different sizes. In other words, the amount of stored energy sources in large seed is higher than that in small seed; thus large seeds produced more vigorous seedlings which might increase the green area for photosynthesis, and consequently, increase the number of tillers and the final yields.

II. Effects of Fertilization on Characteristics of Seeds which were Evaluated in Greenhouse and the Field

Nitrogen fertilization greatly increased the proportion of large seed, average seed size, percent Kjeldahl nitrogen and free sugars. How nitrogen fertilization increases the percent free sugars, is not known yet. A decrease in percent RAC was associated with seeds from nitrogen fertilized plots. Starch is considered to be the major component in the RAC fraction. The above result was in agreement with the finding of Ayre (1940) and McCalla and Corns (1943) that a highly significant negative correlation between the starch and protein contents of barley and wheat existed. This might be interpreted as the transformation

Table 18. Summary of Chemical Composition of Small and Large Seeds
Evaluated in the Field and Greenhouse

Seed from Location	Seed Size	Free sugars		RAC		TAC (a)		Kjeldahl - N	
		%	g/1000 kernels	%	g/1000 kernels	%	g/1000 kernels	%	g/1000 kernels
1	Small	4.3	1.11	64.8	16.7	69.1	17.8	2.11	0.54
	Large	5.1	2.14	66.5	27.9	71.6	30.0	2.12	0.89
2	Small	3.5	0.86	65.0	15.9	68.5	16.8	2.20	0.54
	Large	4.2	1.98	67.2	31.7	71.4	33.6	2.23	1.05
3	Small	2.8	0.62	70.4	15.5	73.2	16.1	1.62	0.36
	Large	2.8	1.08	73.2	28.1	76.0	29.2	1.73	0.66
4	Small	3.4	0.87	71.5	18.4	74.9	19.5	1.95	0.50
	Large	4.3	1.78	70.6	29.2	74.9	30.9	2.10	0.87
5	Small	3.1	0.77	71.6	17.8	74.7	18.6	1.80	0.45
	Large	3.5	1.41	71.0	28.7	74.5	30.1	1.91	0.77

(a) TAC (Total available carbohydrates) = Free sugar content + RAC (Residual available carbohydrates)

of carbohydrates to free amino acids or proteins due to the presence of a sufficient amount of nitrogen in seeds that were obtained from nitrogen fertilized parents.

III. Effects of Parental Fertilization on Early Plant Growth and Yields

During the early growth period, it was observed in the greenhouse that plants grown from seeds from nitrogen fertilized parents seemed to be more vigorous than those from seeds from unfertilized parents. It was reported in the previous section that free sugar and nitrogen contents of seed were significantly increased by nitrogen fertilization. Further, the 1000 kernel weight of a given seed size was slightly greater when nitrogen was applied than when it was not. The variation noted in early growth might be due to the differences in amounts of free sugars and proteins ($N \times 6.25$) stored in seed (Table 19). Both parental nitrogen and phosphorus tended to increase grain and straw yields, but the effect was smaller due to parental phosphorus fertilization. Since parental nitrogen fertilization had a larger effect on seedling vigor, it was expected that the greater grain and straw yields would also be associated with parental nitrogen fertilization.

Although a greater number of seedlings per plot sown with seeds from nitrogen fertilized parents of location 1 was recorded, inverse results were noted for number of healthy heads per plant, 1000 kernel weight and grain yield. Only the effect of parental nitrogen fertilization on straw yield was in the same direction as on emergence. On the contrary, the parental phosphorus fertilization showed exactly reverse results from those found for parental nitrogen fertilization. These unexpected results seem difficult to explain. A highly significant

Table 19. Summary of Chemical Composition of Seeds from
Nitrogen Fertilized and Unfertilized Parents

Seed from Location	N Fert. (a)	Free sugars		RAC		TAC		Kjeldahl - N	
		%	g/1000 kernels	%	g/1000 kernels	%	g/1000 kernels	%	g/1000 kernels
1	0	3.8	1.22	68.1	21.8	71.9	23.0	1.77	0.57
	+	5.6	2.00	63.2	22.2	68.8	24.2	2.37	0.83
2	0	3.5	1.25	67.8	24.3	71.3	25.2	1.93	0.70
	+	4.2	1.51	64.4	23.1	68.6	25.3	2.50	0.90
3	0	2.7	0.80	73.0	21.7	75.7	22.5	1.57	0.47
	+	2.9	0.89	70.6	21.6	73.5	22.5	1.79	0.55
4	0	3.6	1.19	73.1	24.2	76.7	25.4	1.80	0.60
	+	4.1	1.39	68.9	23.4	73.0	24.7	2.26	0.77
5	0	3.1	1.02	73.3	24.0	76.4	25.1	1.67	0.55
	+	3.4	1.11	69.4	22.6	72.8	23.7	2.06	0.67

(a) 0 : No nitrogen fertilization

+ : Nitrogen fertilization

effect of nitrogen parental fertilization on percent smutted heads was observed in seeds from location 1, but there was no consistent effect of parental fertilization on seeds from the other locations.

CONCLUSIONS

1. Nitrogen fertilization tended to increase the proportion of large seed, average kernel weight, nitrogen and free sugar contents of seed, but decreased the percent residual available carbohydrates. There was no effect of nitrogen fertilization on total available carbohydrates of seed.

2. Plant growth and final yield were significantly associated with seed size. Large seed produced a better crop with a lower incidence of loose smut, and greater yields than did small seed.

The results of this study suggest that an increase in final yields of the second generation plants may be gained through the proper fertilization of the previous crops from which seeds are obtained.

3. The differences in the behavior of cut large seed, small seed and large seed during the early growth, might be due to the variation in the energy supply which mainly depends on the amounts of total available carbohydrates and proteins. The behavior of cut large seed shown in the greenhouse studies and the chemical analysis of the seed at least supported the hypothesis that the difference in crops produced by various seed sizes was the result of difference in total available carbohydrates.

4. For a given seed size, parental nitrogen or phosphorus fertilization did not show definite effects on plant growth, nor on subsequent yields. On the basis of this work, insofar as the grain yield is concerned, it will not be worth paying much attention to whether seeds come from fertilized or unfertilized parents except as these treatments change the seed size.

Since germination and subsequent early growth of seed is a very complicated biological phenomenon, it is difficult to assert what is the unique component or reaction which is responsible for the marked differences observed during the early growth stages. In addition to available carbohydrates and nitrogenous compounds present in the seed, fats, mineral components, etc. might be of equal importance during germination. The importance of enzymic activities and the presence of endogenous growth regulators during germination have been reported by plant physiologists. It is also known that the energy sources, enzymic activities, and other compounds are likely to be interdependent. Further studies are required to completely elucidate the differences in the behavior of different seed sizes.

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